

Figure 1

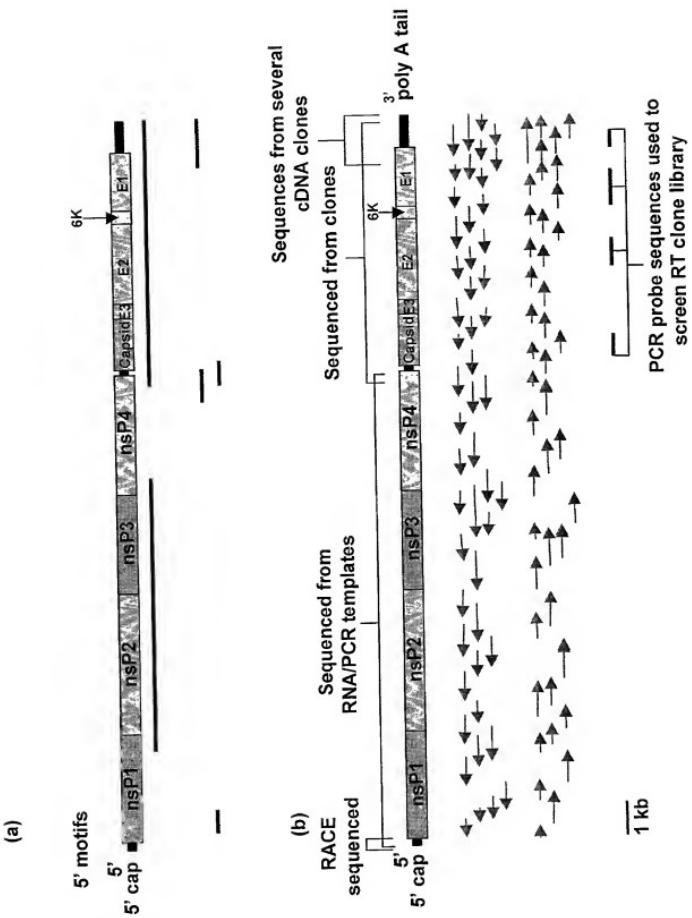


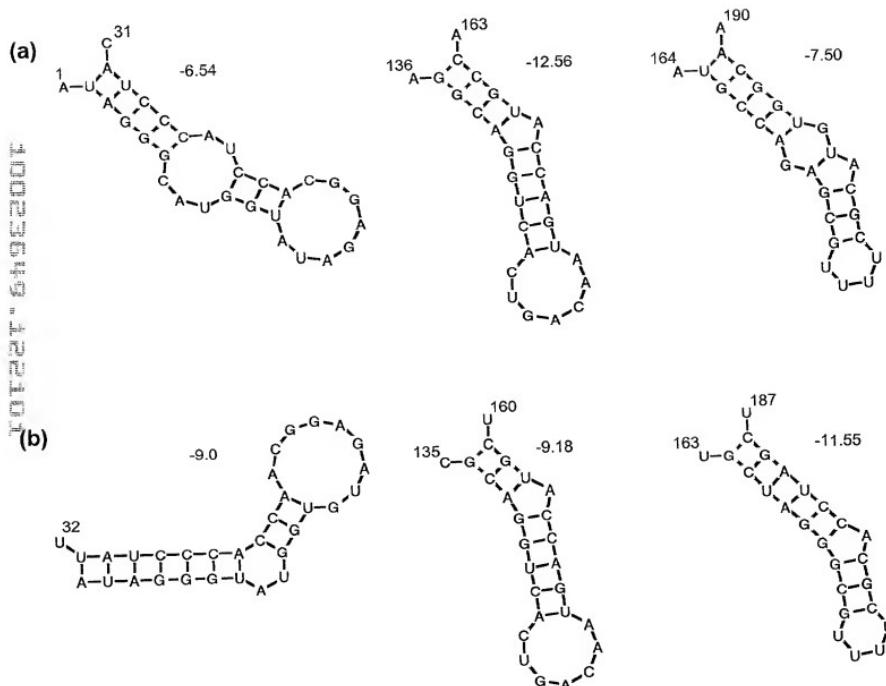
Figure 2 Multiple sequence alignment

(a)	1	A T A G G G C A T G G T A T A G A G G G C A C C T A C C C T A C A A A C [A] A A T C 1 1 1 1	C B A 8 7 7 1 V - 1 6 5 8
	41	G A T C C A A T A T G G A A A G A A T T C A C G T T G A C T T A G A C [G] G C T G A 17 39 37	C B A 8 7 7 1 V - 1 6 5 8 E E E V E E
	81	C A G G C C C A T A T G T C A A G T 57 79 77	C B A 8 7 7 1 V - 1 6 5 8 E E E V E E
	97	T T T G A G A T C G A A G C A A G G C A G G T C A C T G A C A A T G A C C A T G 119 117	7 1 V - 1 6 5 8 E E E V E E
	137	C C A A T G C C A G A G C G T T T T C G C A T G T G G C A A C A A A G C T C A T 159 157	7 1 V - 1 6 5 8 E E E V E E
	177	T G A G G A G C G A A G T C G A C C G G G A C C A A G T T A T C T T G G A C A T 199 197	7 1 V - 1 6 5 8 E E E V E E
(b)		C T C G A T A T G G G C T T C C G C C G T A G G C T C A A G C C T G A T A T A G G G C T T C C G C G T A G G G T C C A G G C T C G A T A T A G G G A T T G C G T C G C C G A A T T A A G	7 1 V - 1 6 5 8 W E E - 5 6 1 4 E E E

a. The 5' terminus of WEE CBA87 (1-97), WEE 71V-1658 (25-240), EEE (1-238) and VEE (1-236) via Clustal module of DNASTar. Areas where sequences differ are boxed.

b. Hypervariable region identified in nsP1. Alignment of WEE 71V-1658 (1420-1449), WEE 1654 (65-94) and EEE (1415-1444) is shown.

Figure 3 Stem loop structures in the 5' NTR

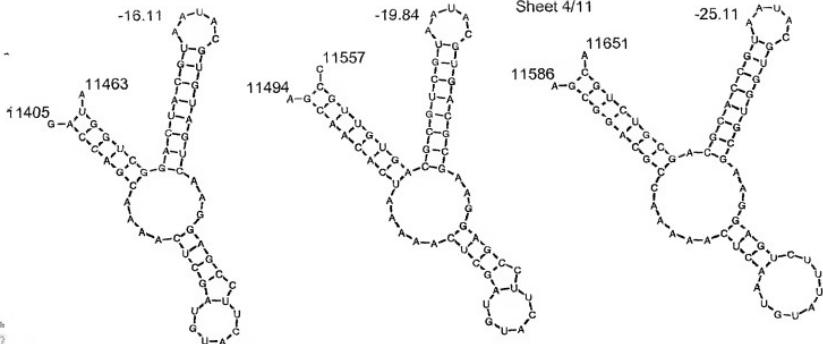


Hairpin structures were identified using the RNA folding program of the Genequest module (DNASTAR).

a. Structures for WEE (CBA87/71V-1658) sequence (1-192).

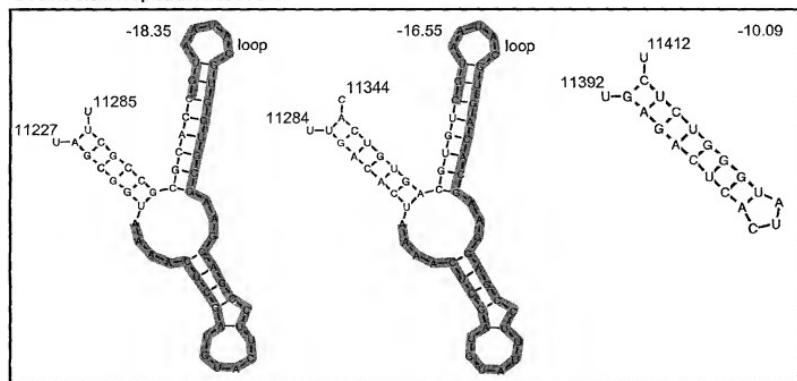
b. Structures for EEE (1-192).

Minimal free energy values are shown for the different structures.

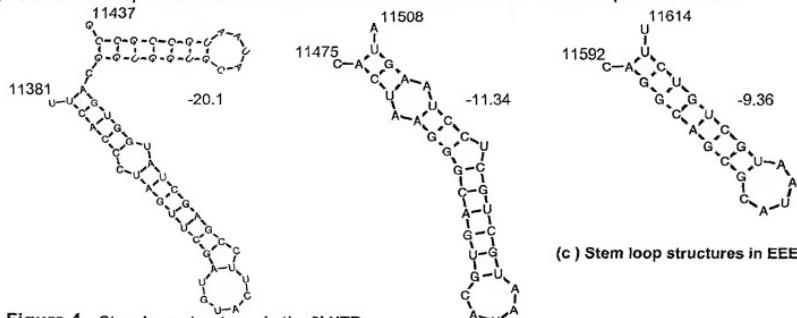


PDB ID: 3WEE

(a) Double stem loop structures in SIN.



(b) Double stem loop structures in 3' NTR of WEE. Residues in the SIN-like 40 nt repeat are shaded.



(c) Stem loop structures in EEE.

Figure 4 Stem loop structures in the 3' NTR

Figure 5 Phylogenetic relationship of the WEE nonstructural region compared to other alphaviruses

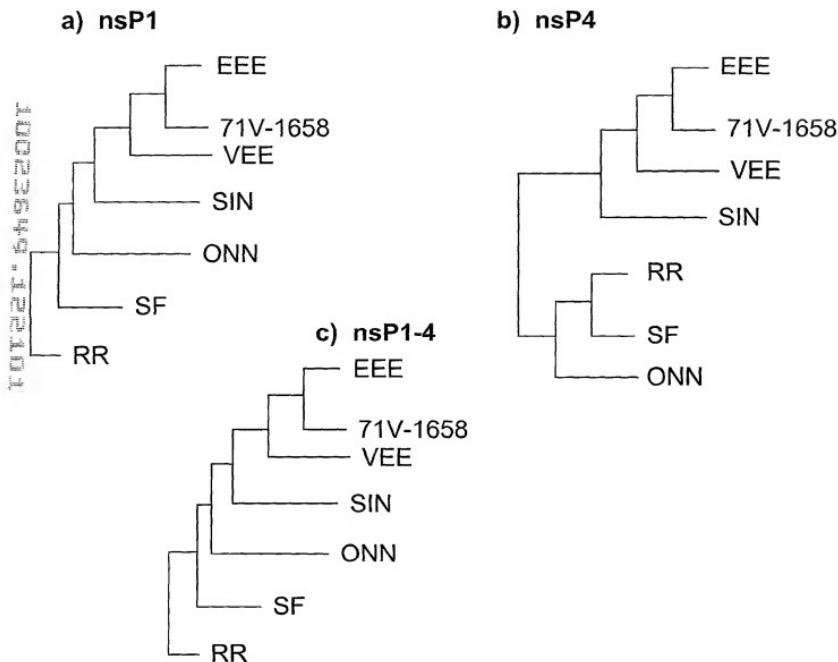
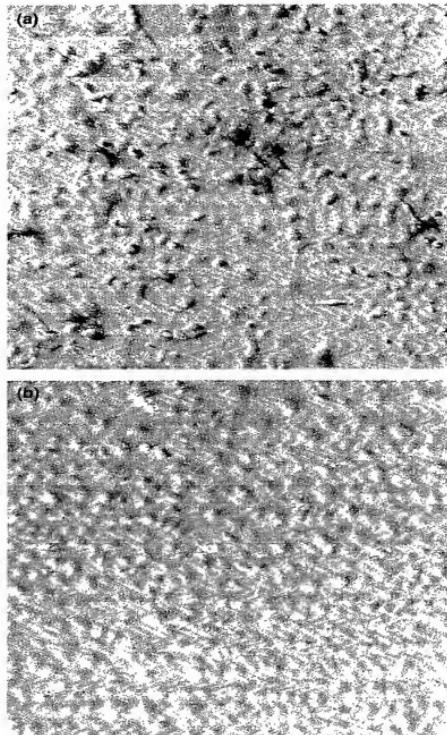


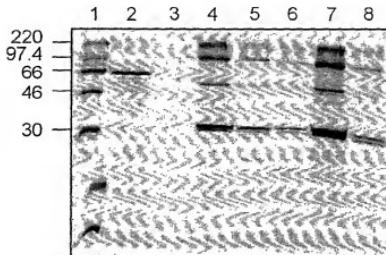
Figure 6 Expression of WEE structural genes in cell culture



One μ g of plasmid DNA was transfected into Vero cells. After 31 hrs incubation, the cells were histochemically stained using a monoclonal antibody to WEE (11D2).

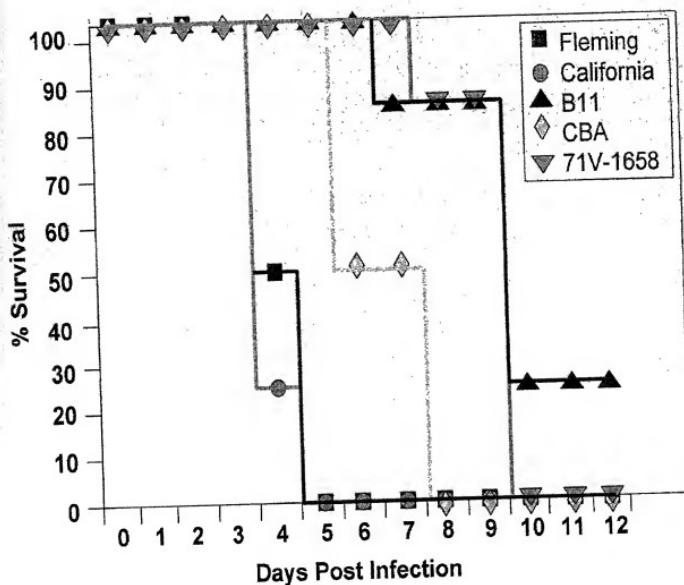
a. pCXH-3; b. pCI (control plasmid).

Figure 7 In vitro transcription and translation of WEE expression vectors

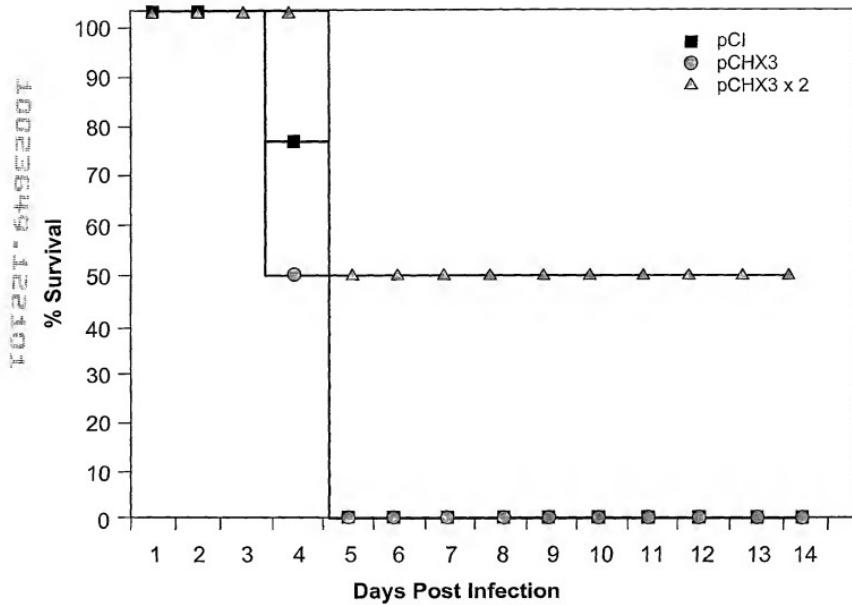


Qiagen purified vectors containing the WEE 26S insert were expressed *in vitro* using the TNT system and [³⁵S]-methionine labelling. Three μ L aliquots of each samples were run by SDS-PAGE on a 12% gel.
Lane: 1) Rainbow ¹⁴C-labelled marker; 2) Luciferase translation control; 3) pVAX; 4) pVHX-6; 5)pCXH-3; 6) pcDWXH-7; 7) pcDWHX-45; 8) pXTR2-4.

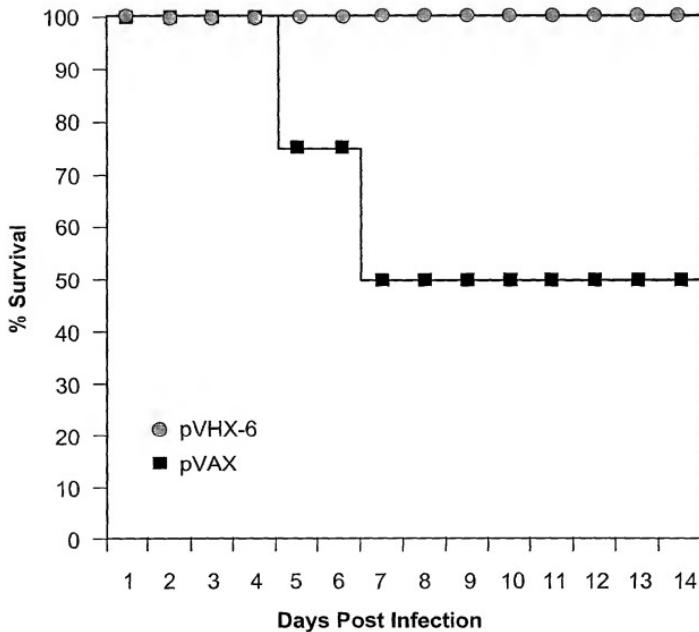
Figure 8 WEE mouse infectivity model



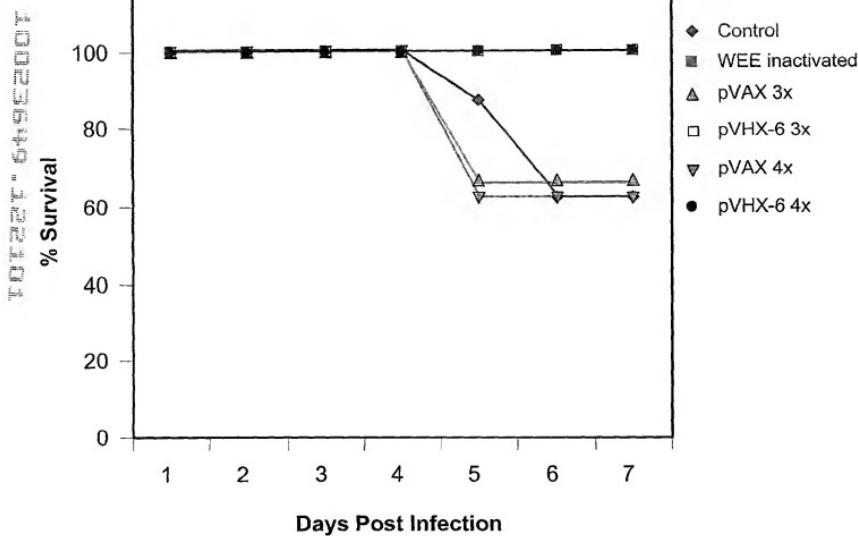
Groups of 4 mice were inoculated intranasally with 50 μL of virus (approximately 10^4 PFU). The mice were monitored for 12 days, and the % survival graphed.

Figure 9 Protection using ballistic delivery of pCXH-3

Groups of 4 mice were immunized with one or two doses ($2 \times 1.25 \mu\text{g}$) of either pCI or pCXH-3. The interval between boosters (2 doses) or challenge was 3 weeks. The mice were challenged intranasally with $50 \mu\text{L}$ of WEE Fleming ($1.25 \times 10^4 \text{ PFU}$). The mice were monitored for 12 days, and the % survival graphed.

Figure 10 Protection using ballistic delivery of pVHX-6

Groups of 4 mice were immunized with four doses ($2 \times 1.25 \mu\text{g}$) of pVAX or pVHX-6. The interval between boosters or challenge was 2 weeks. The mice were challenged intranasally with $50 \mu\text{L}$ of WEE Fleming ($1.25 \times 10^4 \text{ PFU}$). The mice were monitored for 14 days, and the % survival graphed.

Figure 11 Protection using ballistic delivery of pVHX-6

Groups of 5-8 mice were immunized with three or four doses ($2 \times 1.25 \text{ } \mu\text{g}$) of pVAX or pVHX-6. The interval between boosters or challenge was 2 weeks.

The mice were challenged intranasally with $50 \text{ } \mu\text{L}$ of WEE Fleming ($1.7 \times 10^4 \text{ PFU}$). Untreated control and WEE inactivated control (3 doses) groups were also included. The mice were monitored for 14 days, and the % survival graphed.